

AMENDMENTS TO THE CLAIMS

This listing of claims replaces all prior versions, and listings, of claims in the application.

1. (Original) A method of growing spermatogonial stem cells, which comprises growing spermatogonial stem cells by culturing the spermatogonial stem cells using a medium containing glial cell-derived neurotrophic factor (GDNF) or an equivalent thereto and leukemia inhibitory factor (LIF).
2. (Original) The method of growing spermatogonial stem cells of claim 1, wherein the above-described medium further contains at least one of epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF).
3. (Currently Amended) The method of growing spermatogonial stem cells of claim 1 ~~or 2~~, wherein the above-described medium further contains serum.
4. (Currently Amended) The method of growing spermatogonial stem cells of ~~any one of claims 1 to 3~~ claim 1, which further comprises using feeder cells.
5. (Currently Amended) The method of growing spermatogonial stem cells of ~~any one of claims 1 to 4~~ claim 1, which comprises using mammal-derived spermatogonial stem cells.
6. (Currently Amended) The method of growing spermatogonial stem cells of ~~any one of claims 1 to 5~~ claim 1, wherein the above-described glial cell-derived neurotrophic factor (GDNF) or an equivalent thereto is contained at a concentration of 0.5 to 50 ng/ml in the above-described medium.
7. (Currently Amended) The method of growing spermatogonial stem cells of ~~any one of claims 1 to 6~~ claim 1, wherein the above-described leukemia inhibitory

factor (LIF) is contained at a concentration of 10^2 to 10^4 units/ml in the above-described medium.

8. (Currently Amended) The method of growing spermatogonial stem cells of ~~any one of claims 2 to 7~~ claim 2, wherein epidermal growth factor (EGF) is contained at a concentration of 0.5 to 50 ng/ml in the above-described medium.

9. (Currently Amended) The method of growing spermatogonial stem cells of ~~any one of claims 2 to 8~~ claim 2, wherein the above-described basic fibroblast growth factor (bFGF) is contained at a concentration of 0.5 to 50 ng/ml in the above-described medium.

10. (Currently Amended) The method of growing spermatogonial stem cells of ~~any one of claims 3 to 9~~ claim 3, wherein the above-described serum is contained at a concentration of 0.1 to 5(v/v)% in the medium at the start of cultivation of the above-described spermatogonial stem cells, and at a concentration of 0.1 to 20(v/v)% in the medium after passage of the above-described spermatogonial stem cells.

11. (Currently Amended) The method of growing spermatogonial stem cells of ~~any one of claims 4 to 10~~ claim 4, wherein the above-described feeder cells are used by 4 weeks after the start of cultivation at latest.

12. (Currently Amended) Spermatogonial stem cells grown *in vitro* using the growing method of ~~any one of claims 1 to 11~~ claim 1.

13. (Original) A therapeutic agent for infertility containing the spermatogonial stem cells of claim 12.

14. (Original) A medium additive kit that comprises glial cell-derived neurotrophic factor (GDNF) or an equivalent thereto and at least one of epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF), and that is used as added to a culture medium for growing spermatogonial stem cells *in vitro*.

15. (Original) The medium additive kit of claim 12, which further comprises leukemia inhibitory factor (LIF).

16. (Currently Amended) The medium additive kit of claim 14 ~~or 15~~, which further comprises serum.

17. (Original) Use of the spermatogonial stem cells of claim 12 for producing a therapeutic agent for infertility.

18. (Original) A therapeutic method for infertility using the spermatogonial stem cells of claim 12.

19. (Original) A method of producing a non-human animal that forms sperms derived from transplanted spermatogonial stem cells, which comprises the following steps:

a) a step of growing spermatogonial stem cells by culturing the spermatogonial stem cells using a medium containing glial cell-derived neurotrophic factor (GDNF) or an equivalent thereto and leukemia inhibitory factor (LIF);

b) a step of transplanting the spermatogonial stem cells grown in step a) into a seminiferous tubule of an infertile non-human animal to obtain a non-human animal showing spermatogenesis derived from the spermatogonial stem cells.

20. (Original) A method of producing sperm, which comprises the following steps:

a) a step of growing spermatogonial stem cells by culturing the spermatogonial stem cells using a medium containing glial cell-derived neurotrophic factor (GDNF) or an equivalent thereto and leukemia inhibitory factor (LIF);

b) a step of transplanting the spermatogonial stem cells grown in step a) into a seminiferous tubule of an infertile non-human animal to obtain a non-human animal showing spermatogenesis derived from the spermatogonial stem cells;

c) a step of obtaining sperm from the non-human animal.

21. (Original) A method of producing an embryo derived from spermatogonial stem cells, which comprises the following steps:

- a) a step of growing spermatogonial stem cells by culturing the spermatogonial stem cells using a medium containing glial cell-derived neurotrophic factor (GDNF) or an equivalent thereto and leukemia inhibitory factor (LIF);
- b) a step of transplanting the spermatogonial stem cells grown in step a) into a seminiferous tubule of an infertile non-human animal to obtain a non-human animal showing spermatogenesis derived from the spermatogonial stem cell;
- c) a step of obtaining sperm from the non-human animal;
- d) a step of inseminating an ovum with the sperm to obtain an embryo.

22. (Original) A method of producing non-human offspring derived from spermatogonial stem cells, which comprises the following steps:

- a) a step of growing spermatogonial stem cells by culturing the spermatogonial stem cells using a medium containing glial cell-derived neurotrophic factor (GDNF) or an equivalent thereto and leukemia inhibitory factor (LIF);
- b) a step of transplanting the spermatogonial stem cells grown in step a) into a seminiferous tubule of an infertile non-human animal to obtain a non-human animal showing spermatogenesis derived from the spermatogonial stem cells;
- c) a step of obtaining sperm from the non-human animal;
- d) a step of inseminating an ovum with the sperm to obtain an embryo;
- e) a step of transferring the embryo into an oviduct of a pseudopregnant female to obtain non-human offspring.

23. (Original) A method of producing non-human offspring derived from spermatogonial stem cells, which comprises the following steps:

- a) a step of growing spermatogonial stem cells by culturing the spermatogonial stem cells using a medium containing glial cell-derived neurotrophic factor (GDNF) or an equivalent thereto and leukemia inhibitory factor (LIF);
- b) a step of transplanting the spermatogonial stem cells grown in step a) into a seminiferous tubule of an infertile non-human animal to obtain a non-human animal showing spermatogenesis derived from the spermatogonial stem cells;

c) a step of naturally mating the non-human animal with a female to obtain non-human offspring.

24. (Original) A method of producing spermatogonial stem cells incorporating an extraneous gene, which comprises the following steps:

a) a step of growing spermatogonial stem cells by culturing the spermatogonial stem cells using a medium containing glial cell-derived neurotrophic factor (GDNF) or an equivalent thereto and leukemia inhibitory factor (LIF);

b) a step of introducing an extraneous gene to the spermatogonial stem cells grown in step a) to obtain spermatogonial stem cells incorporating the extraneous gene.

25. (Original) A method of producing sperm incorporating an extraneous gene, which comprises the following steps:

a) a step of growing spermatogonial stem cells by culturing the spermatogonial stem cells using a medium containing glial cell-derived neurotrophic factor (GDNF) or an equivalent thereto and leukemia inhibitory factor (LIF);

b) a step of introducing an extraneous gene to the spermatogonial stem cells grown in step a) to obtain spermatogonial stem cells incorporating the extraneous gene;

c) a step of inducing spermatogenesis by transplanting the spermatogonial stem cells to a seminiferous tubule to obtain sperm incorporating the exogenous gene.

26. (Original) A method of producing a transgenic non-human animal, which comprises the following steps:

a) a step of growing spermatogonial stem cells by culturing the spermatogonial stem cells using a medium containing glial cell-derived neurotrophic factor (GDNF) or an equivalent thereto and leukemia inhibitory factor (LIF);

b) a step of introducing an extraneous gene to the spermatogonial stem cells grown in step a) to obtain spermatogonial stem cells incorporating the extraneous gene;

c) a step of inducing spermatogenesis by transplanting the spermatogonial stem cells to a seminiferous tubule to obtain sperm incorporating the exogenous gene;

d) a step of inseminating an ovum with the sperm to obtain a transgenic non-human animal.

27. (Original) The production method of claim 26, wherein the transgenic non-human animal is a knockout non-human animal.